CHAPTER III

MATERIAL AND METHODS

3.1. Material

Rare sugars of D-sorbose were obtained from Kagawa Rare Sugar Research Center, Japan. Threonine (Thr) (with the purity index 99%) was obtained from Cheil Jedang Indonesia, Co. Ltd. ABTS or 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) was obtained from AppliChem, Germany (Lot No.2X001714). While, the instrument needed were digital balance, beaker glass, micropipette, microtubes, dry oven, vortex, refrigerator, pH meter, spectrophotometer and digital colorimeter.

3.2. Methods

3.2.1. Preparation of MRPs Model

The preparation method of MRPs model has been adopted from Yu et al. (2012) with minor modifications. The D-sorbose as a control, 1:1 D-sorbose-Threonine (Sor-Thr) was dissolved in 500 mL of 10 mM carbonate buffer (pH 9) solution. Then, two hundred microliter was transferred to 1.5 mL microtube prior to application for heat treatment. The sample was subjected to 48 h heating process at 50±1.0˚C RH 60% using the controlled dry oven. Measurements were performed every three hour on hour 0 up to 48. After heating treatment, the dried sample were cooled immediately in the air for 1 min, and then kept at 4˚C. The samples to be measured dissolved up to 200 µl of 10 mM phosphate buffer (pH 7). The flow diagram of MRPs model preparation is shown in Figure 3.

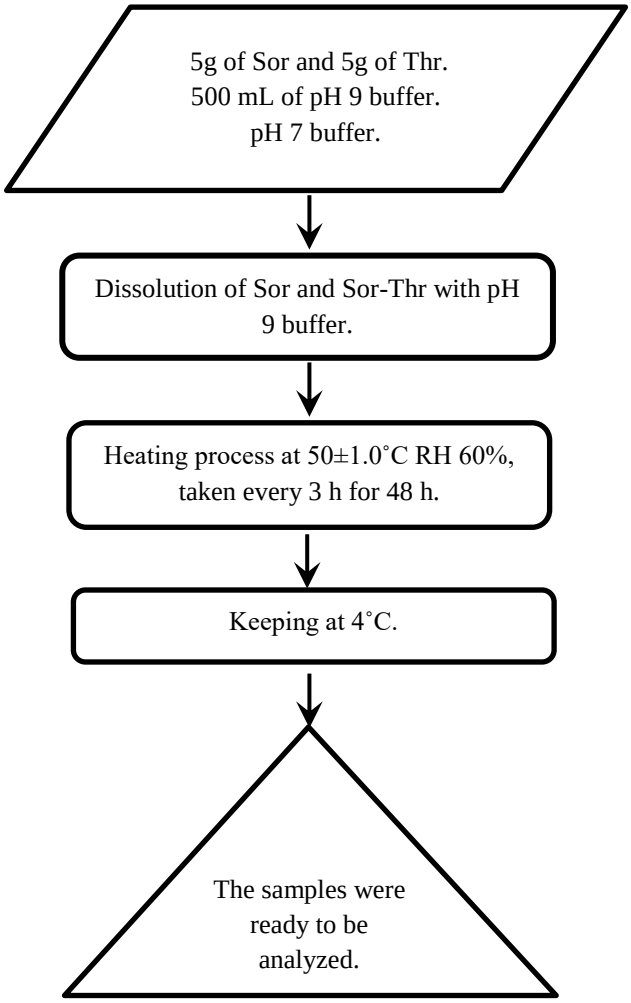

Figure 3. The flow diagram of Maillard reaction products

3.2.2. Browning Intensity

The browning intensity of the MRPs was measured according to the method of Ajandouz *et al*. (2001). The MRPs of Sor and Sor-Thr after heating were dissolved up to 200 µl of phosphate buffer in microtube. Browning intensity of sample was measured by Spectrophotometer (UV-1280; Shimadzu, Kyoto, Japan) at absorbance 420 nm.

3.2.3 Color Development

The MRPs samples of Sor and Sor-Thr after heating were dissolved up to

to 200 µl of phosphat buffer in microtube. The color development or browning index of samples were determined by digital colorimeter TES-135 to obtain the L* a* b* values. The instrument was calibrated with a standard white before measurement, then the browning index was calculated using the equation (2) (Alvarenga *et al*., 2014).

$$x = \frac{a + 1.75\ (L)}{5.645\ (L) + a - 3.012\ (b)} \qquad (1)$$

$$BI = \frac{100\ (x - 0.31)}{0.172} \qquad (2)$$

Here *L, a,* and *b* are the values from digital colorimeter, *x* is the value obtained from equation (1), *BI* is the browning index or the final value. *BI* was obtained from equation (2).

3.2.4. Spectroscopic Measurements

The MRPs samples of Sor and Sor-Thr after heating were dissolved up to to 200 µl of phosphate buffer in microtube. To analyze the spectral formation of MRPs, samples were measured by spectrophotometer at wavelength 190 – 750 nm. This spectroscopic measurements method has been adopted from Jing and Kitts (2004).

3.2.5.. ABTS Radical Scavenging Activity

The ABTS radical scavenging activity of MRPs method has been adopted from Hwang *et al*. (2011) with some modification. 7 mM of ABTS reagent was diluted in 10 ml phosphate buffer pH 7.4. 5 ml of these ABTS solution was added with 88 µl of potassium persulfate. These mixtures were incubated for 16 hour in dark condition at room temperature, to reach a final absorbance of 0.7±0.02 at 734

nm. Then, 1 : 9 of MRPs samples mixed with 90% ethanol, and ABTS stock solution were loaded in spectrophotometer. The percentage inhibition of MRPs scavenging activity from Hwang *et al*. (2011) was calculated in equation (3).

$$y = \frac{A0-A1}{A0} \times 100 \qquad (3)$$

Here A0 is the absorbance with blank and A1 is the absorbance with the sample.

3.2.6. Correlation between Browning Intensity and Scavenging Activity

The browning intensity and the scavenging activity were analyzed for R^2 value. Values of R^2 should be in a range 0 to 1 and it is close to 1, the positive correlation between browning intensity and scavenging activity should be occurred. This correlation analysis method has been adopted from Alvarenga *et al*. (2014).

3.3. Data Analysis

The results were reported in figures and the figures were generated from appendix. The scavenging activity and physical phenomena of MRPs were analyzed using descriptive analysis, and then the significance of correlation between browning intensity and scavenging activity was analyzed using Graphpad Prism version 6.0. This study was adopted from Nilsson *et al*. (2004) and Aon and Colaneri (2001), who has stated that the significant correlation should be if P value <0.0001.